I wish to express my thanks to Dr. J. W. Trevan for his interest, and to Miss M. Llewellyn Smith for her assistance with the calibration of the apparatus and the construction of the scales.

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ON THE INFLUENCE OF TEMPERATURE ON THE RATE OF AGGLUTINATION OF BACTERIA.

ALEXANDER FLEMING.

From the Laboratories of the Inoculation Department, St. Mary's Hospital, London.

Received for publication July 10th, 1928.

THE agglutination reaction as we do it comprises two quite different processes—firstly the union of the agglutinin of the serum with the bacteria, and secondly the clumping of the bacteria. The work of Gaehtgens (1906, 1908) and Gates (1922) on rapid agglutination by means of centrifuging—work which I have confirmed—has shown that the first process, the union of the agglutinin and the bacterium, takes place very rapidly, and that by far the greater portion of the time in the ordinary agglutination reaction is occupied by the sensitized bacteria coming together into clumps. This is clearly shown in the following experiments:

Experiment 1.—Serial dilutions of a paratyphoid B serum were made and a suspension of paratyphoid *B. bacilli* was added to each. Portions of these were centrifuged immediately and after being incubated for different times with the following result:

	Dilutions of serum.					Control.
	1/200.	1/400.	1/800.	1/1600.	1/3200.	
Centrifuged immediately .	+++	+++	++	+	0	0
,, after 20 minutes	+++	+++	++	+	0	0
,, ,, 2 hours .	+++	+++	++	+	0	0

Experiment 2.—Serial dilutions of the serum of a patient suffering from Malta fever were made and a suspension of *M. melitensis* added to each. A portion of the mixture was centrifuged immediately and the rest was incubated at 48°C. for 6 hours. The following readings were then made:

	Dilutions of serum.					Control.
	1/1250.	1/2500.	1/5000.	1/10,000.	1/20,000.	
Centrifuged immediately .	+++	++	++	+	0	0
Incubated at 48° C. for 6 hours	++	++	+	0	0	0

Gates, working with meningococcus and *B. typhosus*, has shown that the temperature makes some difference to the rate of union of the agglutinin with the bacterium, and he calculates that the time and temperature relations are, in regard to meningococcus, roughly those of chemical acceleration.

In this communication I am not, however, concerned so much with the rate of union of the agglutinin as with the influence of temperature on the rate of flocculation.

It is the experience of everyone who has done agglutination tests that the process is hastened by increasing the temperature up to somewhere about 55°C. The following quotation from the report of a Committee of the Medical Research Council on Pathological Methods may be taken to sum up the matter: "It occurs more rapidly as the temperature rises up to an optimum of 55° C., above which the reaction is impaired and soon ceases. It will take place perfectly at room temperature, but requires a much longer time to become manifest."

It is well known that gentle motion accelerates the flocculation and many devices have been introduced to make use of this. It is probable that the virtue of half-immersion in a water-bath lies in the convection currents which are set up.

That motion is more important than temperature has been shown in many ways; simple rocking of serum bacteria mixtures on a slide at room temperature is a method which has been much practised for obtaining rapid agglutination, while the following experiment brings this point out very clearly.

Experiment 3.—Serial dilutions of a patient's serum were made, and to each dilution an equivalent quantity of staphylococcus suspension was added. Two c.c. quantities of each of these mixtures were placed in two series of tubes, one series being placed in a water-bath at 45° C. and the other connected up with a mixing machine at room temperature. At the end of one hour the tubes gave the following readings:

	Dilutions of serum.				Control.
	1/40.	1/80.	1/160.	1/320.	
Mixing machine at 18° C.	+++	+++	++	+	0
Water-bath at 45° C. .	±	0	0	0	0

The mixing machine described by Dr. Ridley (1928) has furnished a convenient method of doing agglutination tests simultaneously at different temperatures, while at the same time giving the same amount of movement to the fluid in each of the tubes. The movement communicated by the mixing machine is so much greater than any convection currents which may be set up that the latter can be neglected, and for practical purposes we may assume that the movement in all the tubes is equal. With the aid of the mixing machine the following experiments were made to determine whether there was any difference in the rate of flocculation at different temperatures when the fluid in the different tubes was moving equally.

Experiment 4.—Serial dilutions of an agglutinating serum were made and to these equivalent quantities of bacterial suspension were added. These were allowed to remain on the bench for 10 minutes to allow of the union of the agglutinin and the bacteria, after which 2 c.c. quantities were transferred to small tubes and connected with the mixing machine, one series being in a water-bath and another series being at room temperature.

Series 1.—Organism used—staphylococcus. Patient's serum.

	Dilutions of serum.							Control.
	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.	1/1280.	
Bench temperature	++	+	0	0	0	—	—	0
Water-bath at 45° C.	++	+	0	0	0	—	—	0
Time, 10 minutes.								
Bench temperature	++	++	++	++	++	+	0	0
Water-bath at 45° C.	++	++	++	++	++	+	0	0
Time, 2 hours.								

Series 2.—Typhoid bacilli. Immune rabbit's serum.

	Dilutions of serum.							Control.
	1/5000.	1/7500.	1/10,000.	1/15,000.	1/20,000.	1/30,000.	1/40,000.	
18° C.	++	+	+	0	0	—	—	0
55° C.	++	++	+	0	0	—	—	0
Time, 15 minutes.								
18° C.	+++	+++	+++	+++	++	+	0	0
55° C.	+++	+++	+++	+++	++	+	0	0
Time, 1 hour.								

A considerable number of experiments have been done with similar results, and it seems clear that a difference of temperature between 18°C. and 55°C. has no influence on the rate of flocculation of the bacteria. There is, however, some difference when flocculation takes place at very low temperatures, as is shown in the following experiment:

Experiment 5.—The technique used was the same as in the previous experiments, except that one set of tubes were immersed in an ice-bath. Typhoid serum and bacilli were used.

	Dilutions of serum.				Control.
	1/500.	1/1000.	1/2000.	1/4000.	
In ice .	+++	+	0	0	0
At 45° C. .	+++	+++	+	0	0

Time, 1 hour.

It is of some interest to record the result of an experiment where the agglutination titre of a serum was estimated by different methods.

Experiment 6.—Serial dilutions of an anti-pneumococcus serum were made and to these were added equal quantities of a suspension of pneumococcus. These were allowed to remain on the bench for 5 minutes to allow of the union of the agglutinin with the bacterium; they were then treated as under with the following results:

	Dilutions of serum.					Control.
	1/20.	1/80.	1/160.	1/320.	1/640.	
Centrifuged for 5 minutes .	+++	+++	+++	±	0	0
1¼ hours kept in motion at room temperature with mixing machine	+++	++	+	0	0	0
1¼ hours at 45° C., half immersed, stationary	+	0	0	0	0	0
3 hours at 45° C., half immersed, stationary	++	±	0	0	0	0

It will be seen that with an organism like pneumococcus, which does not agglutinate very rapidly, one obtains the highest titre after centrifuging for 5 minutes; next comes gentle movement at room temperature, and last of all comes the ordinary method of incubation at a high temperature in a water-bath with the tubes half-immersed. Of course, if the time of incubation had been prolonged to 18 or 24 hours the same titre would probably have been obtained, but an experiment such as this shows the great advantage of the centrifuge method in obtaining satisfactory results in a short time.

CONCLUSIONS.

In agglutination tests, while the rate of union of the agglutinin with the bacterium is hastened to some extent by a rise of temperature (Gates, 1922), the rate of flocculation is not affected by temperature changes between 18° C. and 55° C. There is, however, some delay at temperatures just above freezing-point. It follows, therefore, that the increase in the rate of flocculation with

increase in the temperature when agglutination tests are done in the ordinary way is due to convection currents which are set up in the fluid in the different tubes. These currents would be more marked at the higher temperatures, thus bringing the bacteria more rapidly into contact with each other and so favouring flocculation.

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OBSERVATIONS ON THE MODE OF ACTION OF A VIRICIDAL SERUM.

S. P. BEDSON, M.D., M.Sc.,
Freedom Research Fellow.
From the Hale and Dunn Clinical Laboratories, London Hospital.

Received for publication August 10th, 1928.

IT is a well-established fact that the filtrable viruses are capable of giving rise to specific neutralizing antibodies, but the nature and mode of action of these antibodies still remains somewhat obscure. Although there is some evidence, in the case of a rabicidal serum at any rate (Murillo, 1911; Kondo, 1922), that the time and temperature to which the mixtures of virus and serum are exposed are important factors in neutralization experiments, this is not generally conceded and there are those who hold that no interaction takes place between neutralizing antibody and virus *in vitro*, the viricidal phenomenon having as its essential setting the living tissues or the interior of living cells. Such a conception implies a fundamental difference between the mode of action of the neutralizing antibody of a viricidal serum and those antibodies with which we are familiar in bacteriological work. There are, of course, considerable difficulties inherent in the demonstration of what actually takes place when a virus is mixed with its specific viricidal serum. One cannot separate the two as one could in an absorption experiment with one of the visible bacteria, so that this method is not available for determining whether a filtrable virus unites with its neutralizing antibody in the test-tube.